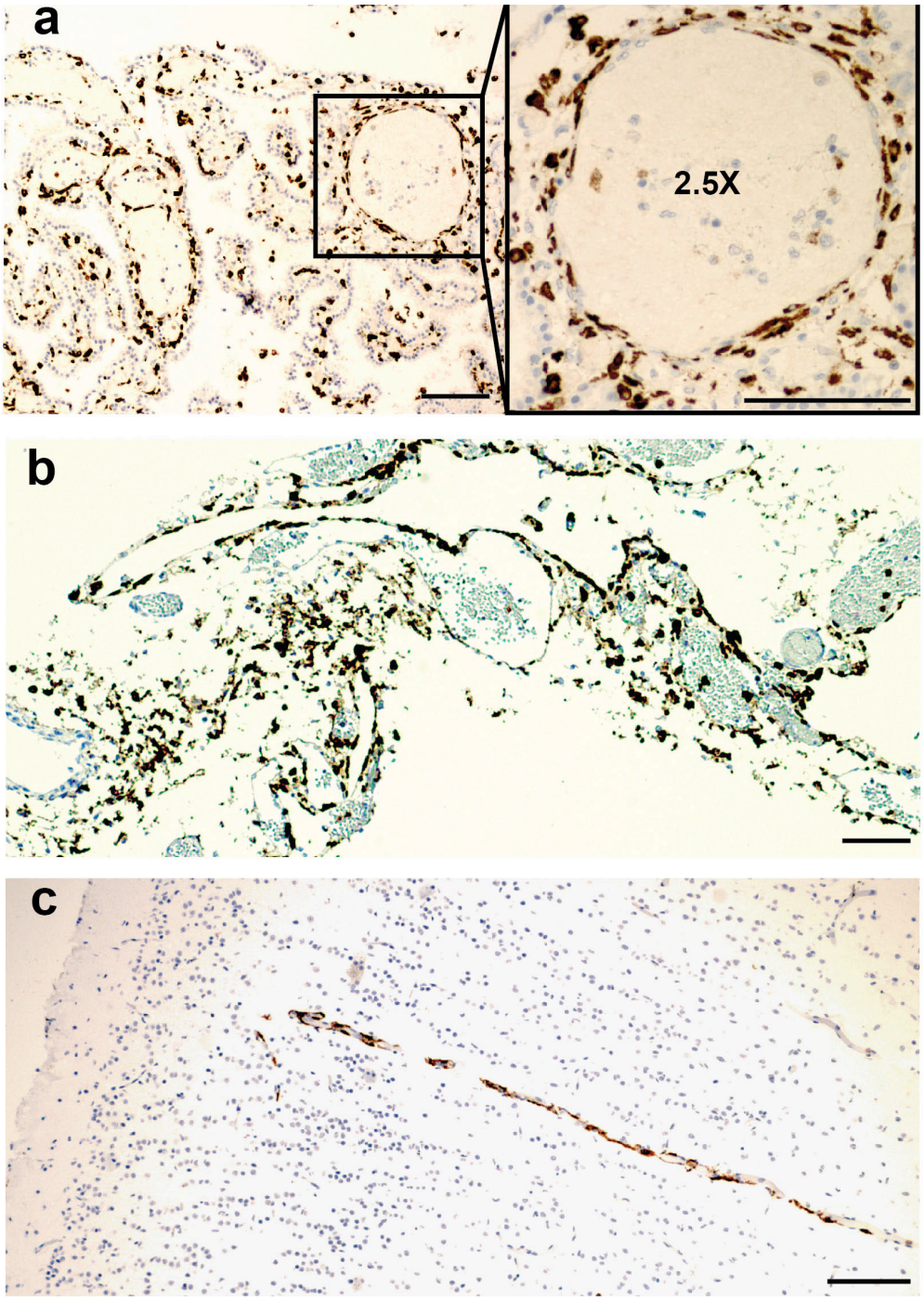
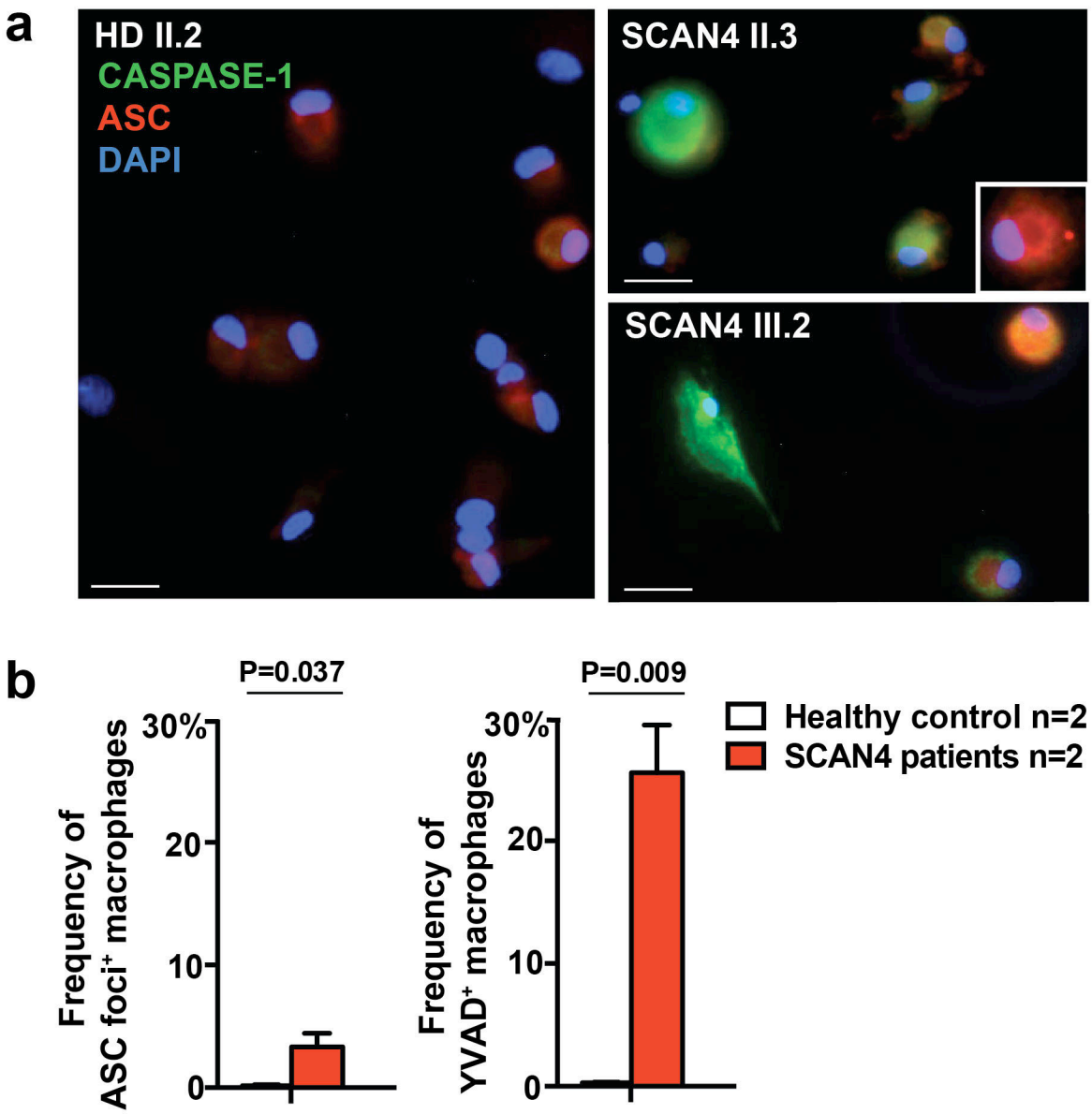


Supplementary Figure 1

Inflammatory cells infiltrating the central nervous system of deceased patient III.3 are mostly activated ($CD163^+$) macrophages.

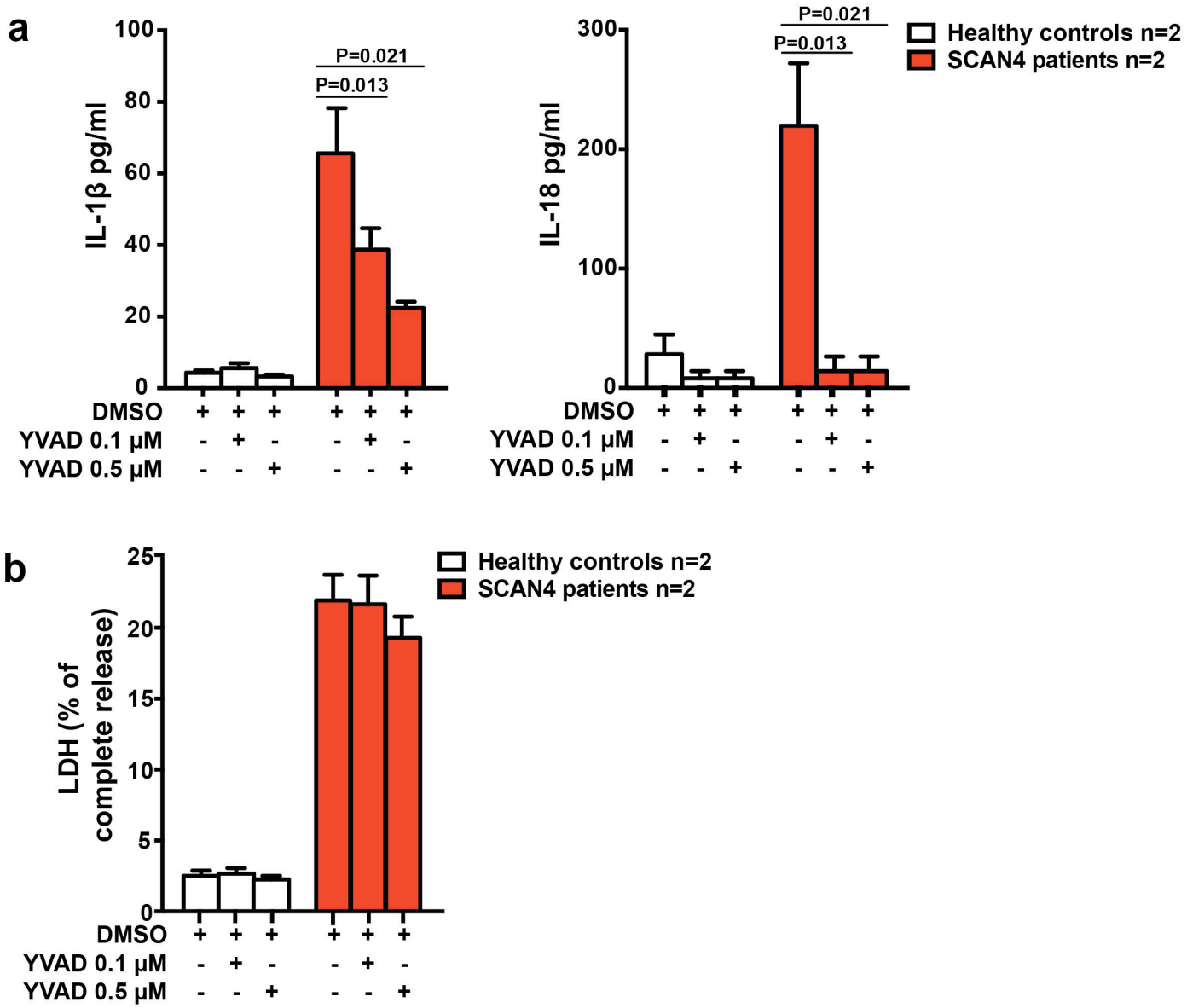
(a) Immunohistochemical staining of the patient's choroid plexus shows the presence of numerous $CD163^+$ macrophages. A vessel cut in cross-section reveals perivascular infiltration with $CD163^+$ macrophages (inset). $CD163^+$ macrophages also diffusely infiltrate the (b) leptomeninges and the (c) cortical perivascular space. Original magnification 200X (for a and b), 100X (for c) and 400x (for inset). Scale bars, 200 µm.



Supplementary Figure 2

SCAN4 macrophages show constitutive activation

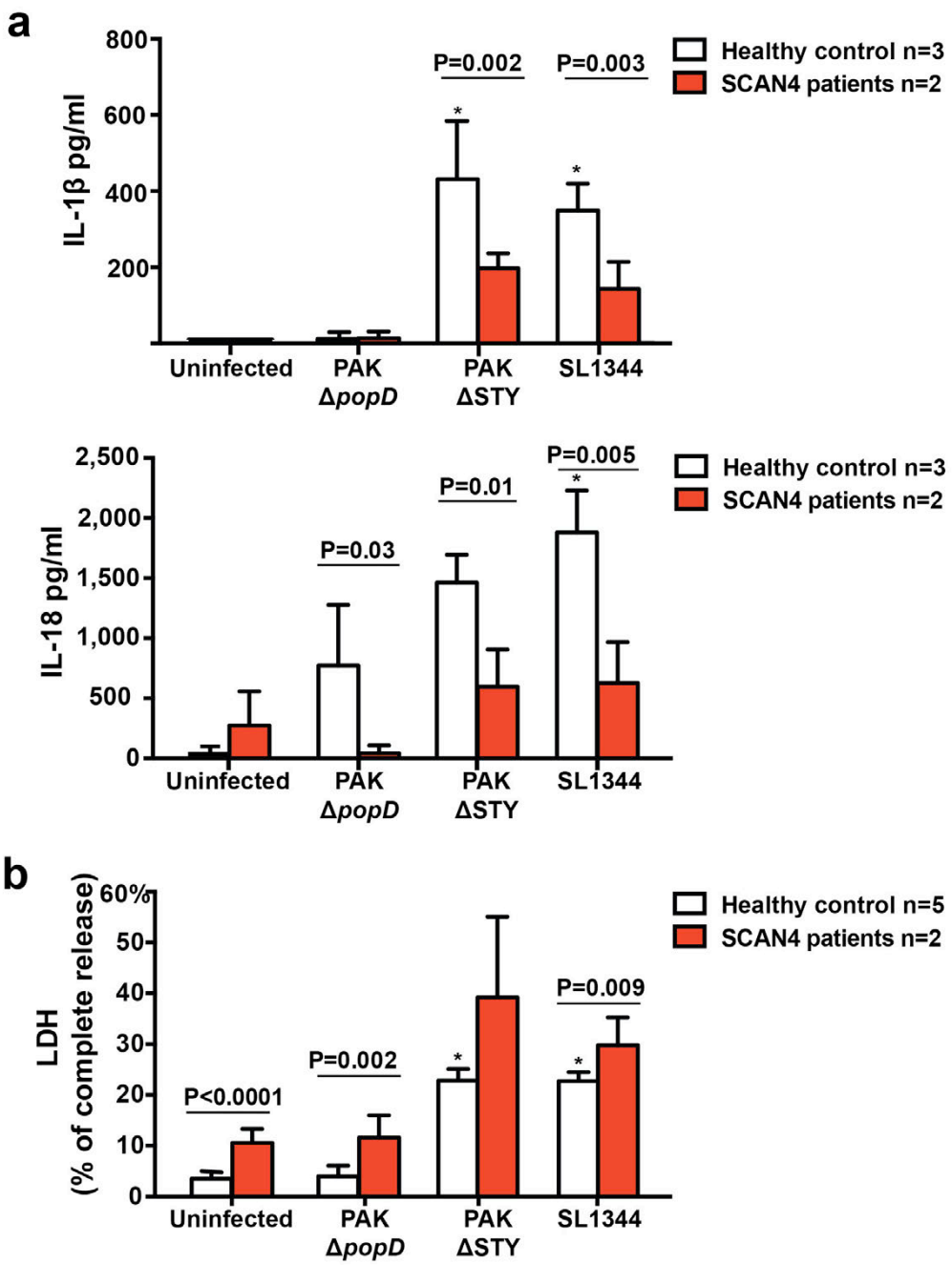
(**a**, right panels) A fraction of uninfected macrophages from SCAN4 patients II.3 and III.2 but not from a representative healthy donor (left panel) spontaneously formed ASC foci (red puncta, inlay) and stained biotin-YVAD-CMK positive (green cytoplasm). (**b**) Frequencies of biotin-YVAD-CMK positive and ASC foci positive macrophages from SCAN4 patients (n=2) and healthy controls (n=2) visualized across multiple microscopic fields. Scale bars, 20 µm. Bar graph shows mean ± S.E.M. Significance by unpaired Student's t-test is indicated.



Supplementary Figure 3

In SCAN4 macrophages, constitutive IL-1 family cytokine secretion, but not spontaneous cell death, is dependent upon the caspase-1 catalytic site that processes cytokines

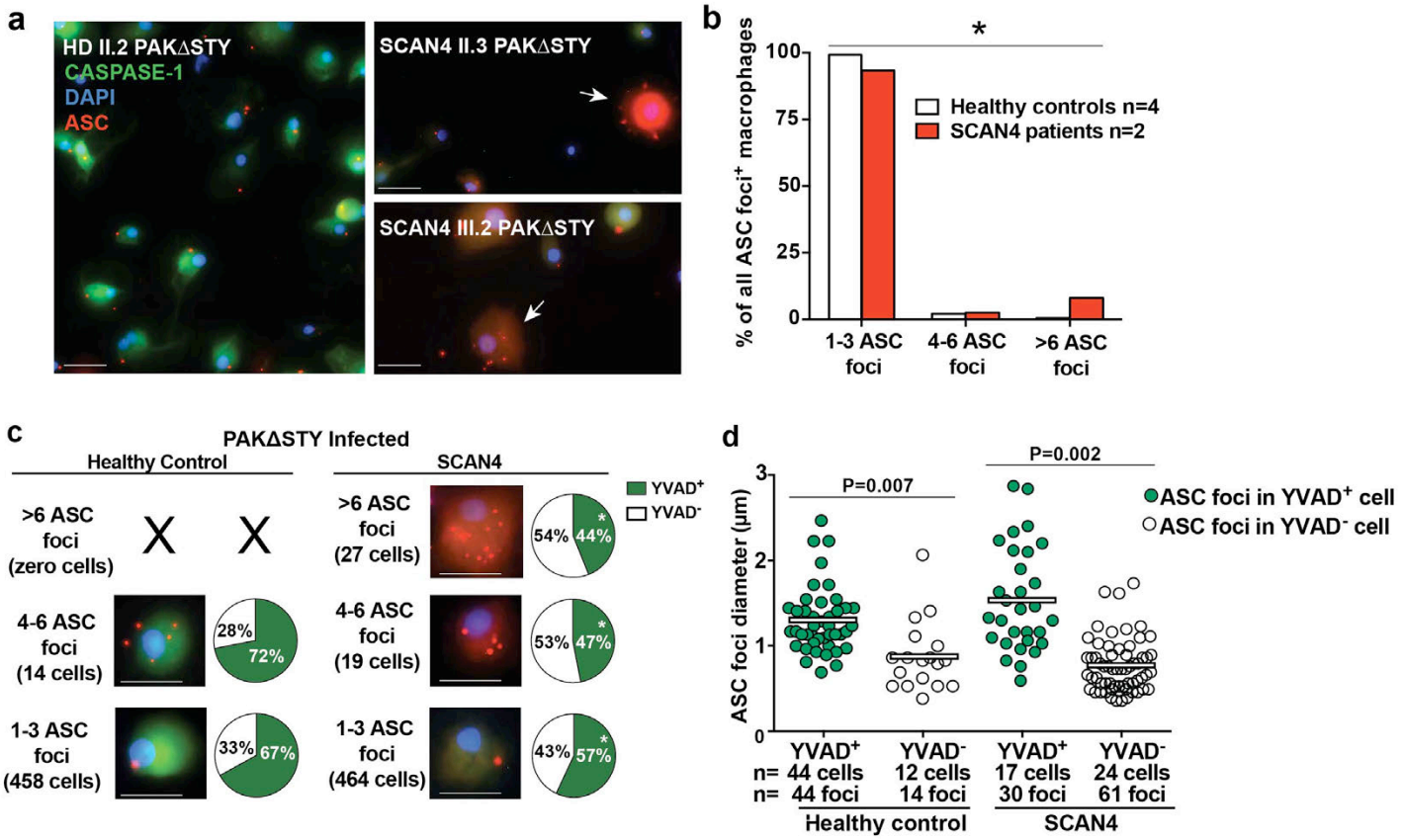
(**a**) IL-1β and IL-18 secretion from monocyte-derived macrophages from SCAN4 patients (II.3 and III.2) cultured for 18 hours in media containing low-dose LPS (1ng/ml) was attenuated by the caspase-1 inhibitor Z-YVAD-FMK (0.1 or 0.5µM) relative to a vehicle control (DMSO). (**b**) Cell death associated LDH release by SCAN4 macrophages over the same 18-hour period is not affected by Z-YVAD-FMK. LDH release is reported relative to cells lysed with Triton X-100 (0.1%). Bar graphs indicate mean supernatant values from experiments performed in duplicate or triplicate and error bars represent ± S.E.M. Significance by paired Student's t-test is indicated.



Supplementary Figure 4

SCAN4 macrophages infected with pathogenic strains result in increased cell death yet blunted IL-1b and IL-18 production

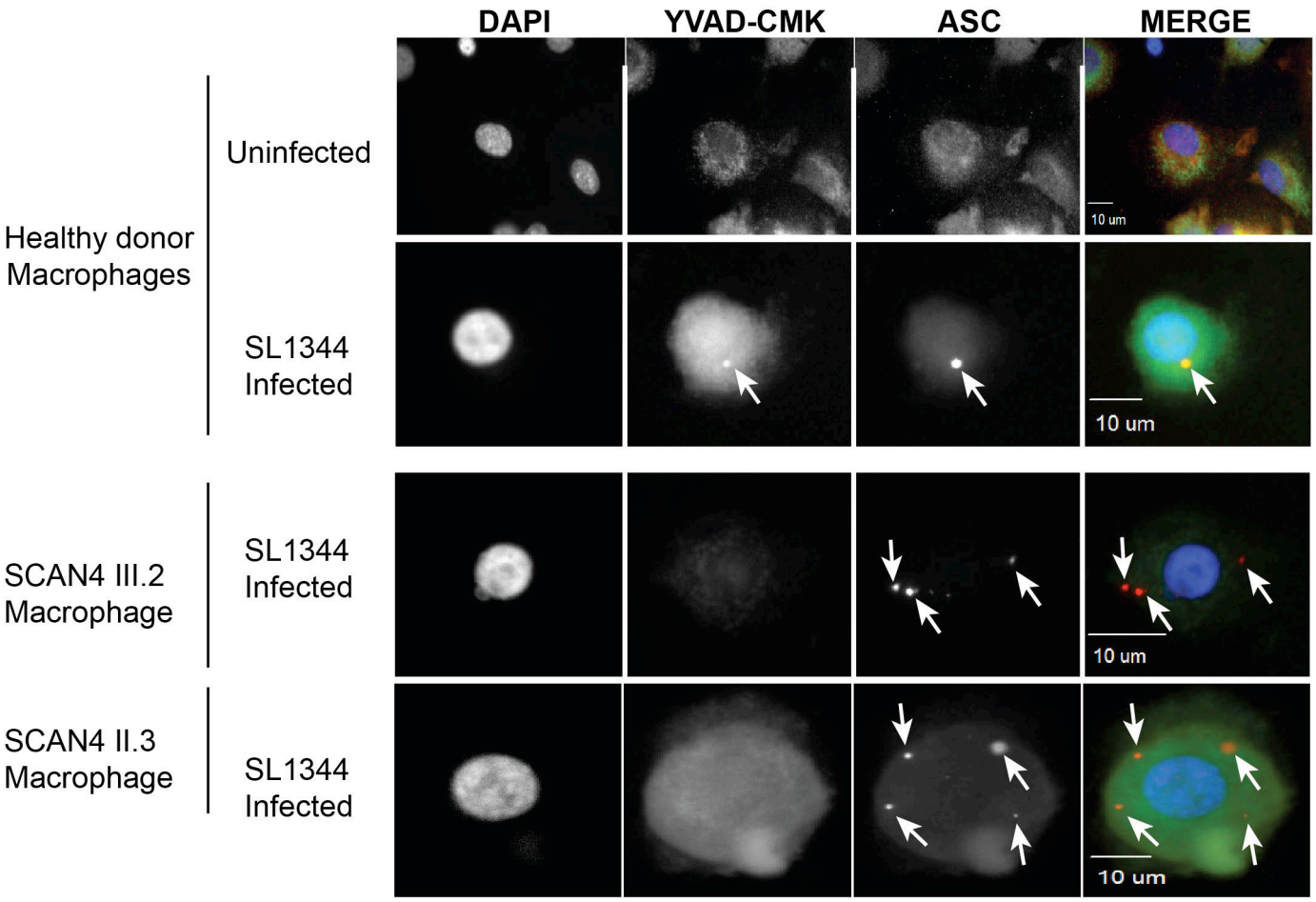
(**a**) Monocyte LPS and infected with pathogenic strains PAKΔSTY and SL1344 or non-pathogenic PAKΔ*popD* as described in Methods. Secretion of (**a**) IL-1β, IL-18 and (**b**) LDH release was measured in three independent experiments. Significant differences between subject groups assessed by unpaired Student's t-test with P values noted. Significant differences between wildtype cells infected with PAKΔSTY or SL1344 versus wildtype cells infected with PAKΔ*popD* assessed by paired Student's t-tests and noted by * when P<0.05.



Supplementary Figure 5

SCAN4 macrophages infected with *P. aeruginosa* strain PAKΔSTY form increased numbers of ASC foci but show limited activation of caspase-1

(a) The majority of PAKΔSTY infected monocyte-derived macrophages from a representative healthy control (HD II.2, the mother of SCAN4 patient III.2) display features of conventional macrophage activation including biotin-YVAD-CMK staining (green cytoplasm) and a limited number (1-3) of ASC foci (red puncta) per cell (left panel). Many PAKΔSTY infected macrophages (white arrows) from SCAN4 patients II.3 and III.2, but not healthy controls, display numerous (>6) ASC foci but show limited staining for biotin-YVAD-CMK (right panel). (b) Quantitation of ASC foci in 472 PAKΔSTY infected healthy control macrophages and 510 PAKΔSTY infected patient macrophages. Patient cell distribution was significantly different (P<0.0001) from healthy control by chi-square testing. (c) Representative biotin-YVAD-CMK staining in PAKΔSTY infected healthy control or SCAN4 macrophages from groups with either 1-3, 4-6 or >6 ASC foci/cell. Pie charts display the frequency of biotin-YVAD-CMK positive cells per group. (d) Diameter of ASC foci in biotin-YVAD-CMK positive macrophages (filled green circles) and biotin-YVAD-CMK negative macrophages (unfilled circles) from healthy control and SCAN4 patients II.3 and III.2. Mean foci diameter displayed as white bars. Scale bars, 20 μm. * patient cell distribution is significantly different (P=0.0012) from healthy control cell distribution by chi-square testing. Significance by unpaired Student's t-test is indicated (for d).



Supplementary Figure 6

ASC foci in *S. typhimurium* strain SL1344 infected SCAN4 macrophages do not colocalize with aggregates of caspase-1

Uninfected macrophages from healthy controls (top row) do not form ASC foci (arrows) whereas *S. typhimurium* strain SL1344 infected healthy control macrophages (second row) stain diffusely with biotin-YVAD-CMK and form aggregates of caspase-1 (arrows) and ASC foci (arrows) that co-localize in merged images (arrows). SCAN4 macrophages diffusely staining biotin-YVAD-CMK negative (third row) or positive (fourth row) form multiple ASC foci that do not co-localize with aggregates of caspase-1. DAPI staining as labeled and also in merged images. All images 60X except uninfected (top row) which is 100x. Scale bars sized as indicated.